Cloning, sequence analysis, and expression of the genes encoding lytic functions of Bacteriophage ϕg1e

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Abstract

The lysis genes of a *Lactobacillus* phage ϕg1e were cloned, sequenced, and expressed in *Escherichia coli*. Nucleotide sequencing of a 3813-bp ϕg1e DNA revealed five successive open reading frames (ORF), *Rorf50*, *Rorf118*, *hol*, and *lys* and *Rorf175*, in the same DNA strand. By comparative analysis of the DNA sequence, the putative *hol* product (holin) has an estimated molecular weight is 14.2 kDa, and contains two potential transmembrane helices and highly charged N- and C-termini, resembling predicted holins (which are thought to be a cytoplasmic membrane-disrupting protein) encoded by other phages such as mv1 from *Lactobacillus bulgaricus*, ϕadh from *Lactobacillus gasseri*, as well as monocins from *Listeria*. On the other hand, the putative ϕg1e *lys* product (lysin) of 48.4 kDa shows significant similarity with presumed muramidase, known as a cell wall peptidoglycan-degrading enzyme, encoded by the *Lactobacillus* phage mv1 and ϕadh, the *Lactococcus lactis* phage ϕLC3, and the *Streptococcus pneumoniae* phages Cp-1, Cp-7 and Cp-9. When expressed in *E. coli*, the ϕg1e lysin and/or holin decreased the cell turbidity significantly, suggesting that the ϕg1e *hol-lys* system is involved in cytolytic process.

Keywords: Bacteriophage ϕg1e; Lactic acid bacteria; Lysin; Holin; Muramidase; DNA sequence

1. Introduction

Prevalence of lysogeny in various lactic acid bacteria has been reported (Davidson et al., 1990). For release of progeny particles from the host cell, such lysogenic phages appear to encode a set of enzymes, which degrade the host cell envelope consisting of several structural components such as peptidoglycan layer and cytoplasmic membrane, and the lysis genes seem to be expressed under a complicated control, as in the coliphage lambda system (for a review, see Young, 1992).

In several phages, the cytolytic process has been presumed to depend upon two phage-encoded lysis proteins. The one known as a holin inflicts lesions in the cytoplasmic membrane, through which the other lysis protein termed lysin can be efficiently released to the periplasm. The lysins belong to a group of murein-degrading enzyme (muramidase), and have been divided into several subgroups: lysozyme, glycosidase, amidase or endopeptidase (for reviews, see Young, 1992; Sable and Lortal, 1995).

Several presumptive lysins and holins have been reported from phages of lactic acid bacteria: ϕadh from *Lactobacillus gasseri* (Henrich et al., 1995), mv1 and mv4 from *Lactobacillus bulgaricus* (Boizet et al., 1990), and ϕLC3 (Birkeland, 1994) and Tuc2009 (Arendt et al., 1994) from *Lactococcus lactis*.

In contrast to coliphage lambda (Young, 1992; Campbell, 1994) and P2 (Ziermann et al., 1994), molecular details on the lytic pathway of the phages from lactic acid bacteria are still insufficient; thus structure, expression, function and enzymatic feature on the phage lysis genes mostly remain to be elucidated (Young, 1992; Sable and Lortal, 1995).

Recently, we isolated a new *Lactobacillus* phage, termed ϕg1e, which contains an about 42.5-kbp DNA, and identified the major phage capsid genes (Kakikawa et al., 1996). In this report, we described structures of ϕg1e genes related to cytolytic process, and their expressions in *E. coli*.

2. Materials and methods

2.1. Bacteria, phage, and plasmids

The lysogenic *Lactobacillus* strain G1e and its temperate phage ϕg1e were originally isolated in our laboratory (Kakikawa et al., 1996), and were propagated in M17 broth (Terzaghi and Sandine, 1975). The *E. coli* vector plasmids pUC18, pUC118, and pUC119, and their host XL1-blue and CK111 *recA lac*$^+$ were from our laboratory stock (Kodaira et al., 1992), and were propagated in LB or 2YT broth (Sambrook et al., 1989).

2.2. Analysis of DNA

Cloning and sequencing analyses of ϕg1e DNA were carried out essentially as described by Kakikawa et al. (1996). Recombinant plasmids were introduced into *E. coli* XL1-blue by Ca^{2+}-dependent transformation or electroporation (Taketo, 1988). For DNA sequencing, various deletion clones were constructed from several series of restriction library from ϕg1e DNA (Kakikawa et al., 1996) by exonuclease digestion (Kodaira et al., 1994a). The DNA sequence was determined by the chain termination method (Sanger et al., 1977). All other procedures were performed as described previously (Kodaira et al., 1994b).

2.3. Expression of ϕg1e genes in E. coli

E. coli XL1-blue carrying a recombinant plasmid, which contains the ϕg1e genes, was grown at 25 °C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.3, the growth temperature was shifted up to 37 °C with or without addition of isopropyl-β-D-thiogalactopyranoside (IPTG) (final concentration, 1 mM), and the cell growth was monitored by measuring the change in A_{660}. Activity of β-galactosidase was determined as follows: *E. coli* CK111 carrying a plasmid pL19PPR was grown at 25 °C in LB medium containing ampicillin (60 μg/ml), and shifted to 37 °C when the A_{660} of the culture had reached 0.3. At intervals, an aliquot of the culture was removed, centrifuged, and the supernatant fluid was reserved. β-Galactosidase activity in the supernatant was assayed at 37 °C using O-nitrophenyl-β-galactoside (0.7 mM) in 0.1 M sodium phosphate buffer (pH 7.3). The activity was followed by measuring A_{420} of nitrophenol released.

All other methods for in vivo experiments were as described previously by Kodaira et al. (1992).

2.4. Enzymes and biochemicals

Restriction enzymes, phage T4 DNA ligase, and alkaline phosphatase (calf intestine) were purchased from Takara Shuzo (Kyoto) and Nippon Gene (Toyama). Buffers for each enzymes were as recommended by the manufacturers.

[α-^{32}P]dCTP was from NEN. All other materials were as described previously by Kodaira et al. (1992).

3. Results and discussion

3.1. Sequence analysis of the phage ϕg1e lysis genes

Bacteriophage ϕg1e induced from a lysogenic *Lactobacillus* strain Gle has a double-stranded DNA of approximately 42.5 kbp. Recently, we have constructed a ϕg1e restriction map, and determined the nucleotide (nt) sequences of four structural genes, G, P, B, and O, which code for major capsid proteins (Kakikawa et al., 1996).

Downstream of the gene O, the extended sequencing analysis of a 3813-bp DNA revealed five ORFs *Rorf50* (153 bp), *Rorf118* (357 bp), *hol* (429 bp), *lys* (1329 bp), and *Rorf175* (528 bp), in addition to a minor ORF *Rorf94* (285 bp) and one truncated ORF *Rorf148* (Fig. 1). In Fig. 2, the DNA sequence of 3813 bp containing these ORFs (gene O through Rorf 148) is presented together with their predicted amino acid (aa) sequences. All the ORFs are encoded on one strand, and preceded by a potential ribosome binding sequence, which is in good agreement with those of several *Lactobacillus* (5′-AGGAGG-3′, Pouwels and Leer, 1993) and *Lactococcus* (5′-AGAAAGGAGGT-3′, Ludwig et al., 1985; Schouler et al., 1994) genes.

As described below, the two potential genes *hol* and *lys* are probably involved in bacterial cell lysis, and their putative products were termed holin and lysin, respectively. *hol* begins with ATG at nt position 978 (Fig. 2), and codes for a basic protein (holin) of 142 aa, whose estimated MW and p*I* are 9450 and 14.2, respectively. On the other hand, *lys* starts with GTG located at nt position 1390 (Fig. 2), and its putative product (lysin) of 422 aa (48.4 kDa) is also basic (with 9.70 of pI). *lys* has one more potential start codon (ATG) located at nt position 1432, although its ribosomal binding sequence (5′-GGA-3′) is very short (Fig. 2).

In other three ORFs *Rorf50*, *Rorf118*, and *Rorf175* as well as a minor ORF *Rorf94*, their putative products show no significant similarities to other proteins, and their functions in the ϕg1e development have not been ascertained. In gp*Rorf175*, however, its middle regions shows a limited resemblance (30% identical, 40% similar) to a lysis protein, named LysA, of coliphage P2, which has been predicted to play a role in the correct timing of lysis, although nonessential (Ziermann et al., 1994). gp*Rorf50* (50 aa) is very acidic (with 3.85 of pI), and has a leucine-rich hydrophobic N-terminus (Fig. 2).

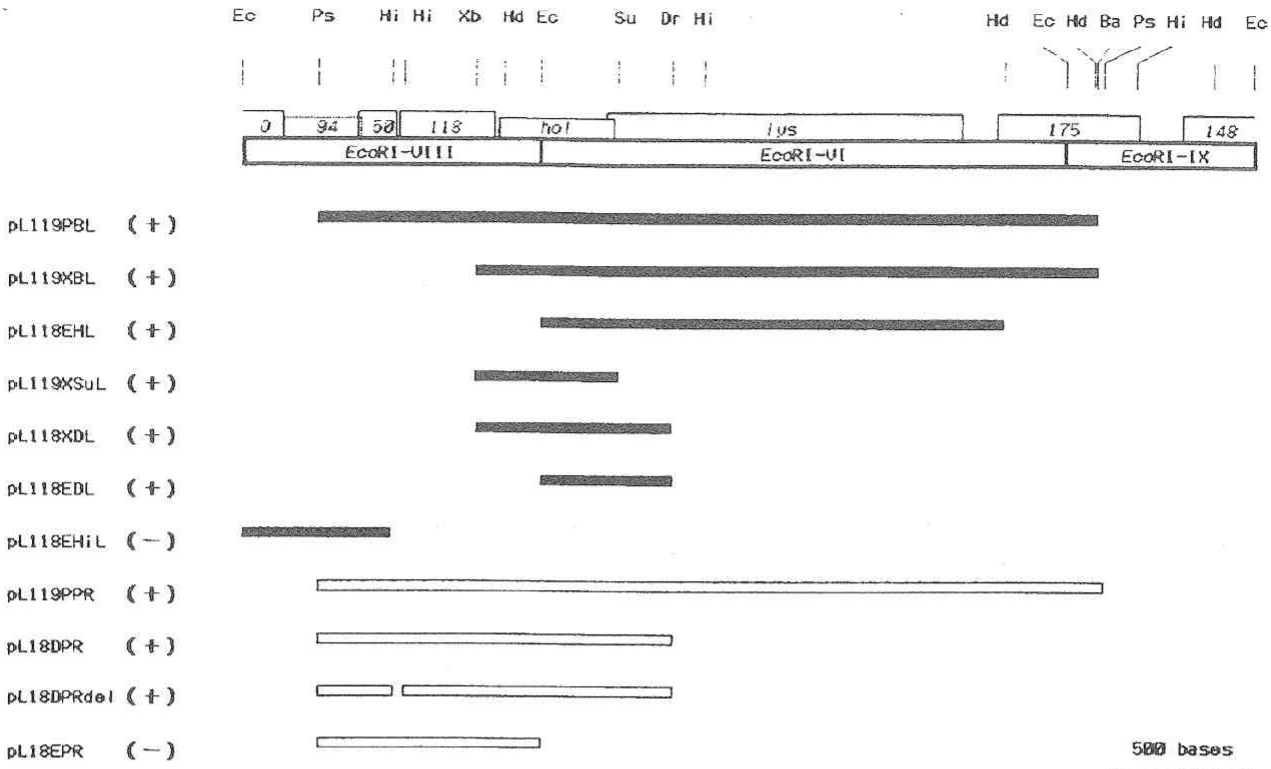


Fig. 1. Physical map of the ϕg1e lytic genes. The recombinant plasmids and their predicted lytic phenotypes are indicated. The construction of the recombinant plasmids is indicated in Table 1. The closed boxes show the ϕg1e DNA inserts cloned under the *lacZ'* promoter. The lytic phenotype (+ or −) was predicted by the decrease of *E. coli* cell turbidity as shown in Fig. 6. Ba, *Bam*HI; Ec, *Eco*RI; Dr, *Dra*I; Hd, *Hind*III; Hi, *Hinc*II; Ps, *Pst*I; Su, *Sau*3AI; Xb, *Xba*I.

On the other hand, gp*Rorf148* has an arginine-rich N-terminus, which somewhat resembles the arginine-rich motif found in antiterminator proteins of coliphages lambda and ϕ21, and *Salmonella typhimurium* phage P22 (for a review, see Burd and Dreyfuss, 1994).

3.2. *Structural characteristics of the ϕg1e lysin*

ϕg1e lysin shares significant similarity with presumptive muramidases encoded by the *Lactococcus lactis* phage ϕLC3 (Birkeland, 1994) and Tuc2009 (Arendt et al., 1994), the *Lactobacillus bulgaricus* phage mv1 (Boizet et al., 1990) and mv4 (Dupont et al., 1993), the *Lactobacillus gasseri* phage ϕadh (Henrich et al., 1995), the *Streptococcus pneumoniae* phage Cp-1, Cp-7 and Cp-9 (Garcia et al., 1990), the coliphage lambda (Sanger et al., 1982), and the fungus *Chalaropsis* (Fouche and Hash, 1978). For example, the similarity between the lysins of ϕg1e and ϕLC3 is 31% identical and 52% equivalent.

In *Chalaropsis*, the two acidic residues Asp^{6} and Glu^{33} have been estimated to be pivotal for the muramidase activity (Fouche and Hash, 1978). N-terminus of ϕg1e lysin has also identical aa residues, Asp^{36} and Glu^{67} (Fig. 3A), suggesting that an active center of ϕg1e lysin is localized in the N-terminus (see Fig. 6C). Middle region of ϕg1e lysin (in about 90-aa region) contains four homologous regions with that of other phages such as ϕadh, mv4, ϕLC3, and Cp-1 (Fig. 3B). Henrich et al. (1995) have reported that these four regions, termed motif A, B, C, and D, may play some structural or functional roles. C-terminal half of ϕg1e lysin is different from other lysins in both length and aa sequence (see Fig. 5). In the C-terminus of 43 aa, ϕg1e shares sequence similarity (19 aa identical, see Fig. 3C) with ϕLC3. In ϕLC3 (Birkeland, 1994), the 43-aa region has been suggested to be a functional domain involved in binding to the bacterial cell wall, possibly through recognition of the peptidoglycan moiety. Thus, ϕg1e lysin seems to be composed of three modules: the N-terminus (termed module DE) functioning as an active center of the enzyme; the middle region (module ABCD); the C-terminal half (module MEM) acting as a recognition site(s) of cell wall (Fig. 5). As pointed out by Birkeland (1994), exchange of these modular units may be an important principle in lysis protein evolution.

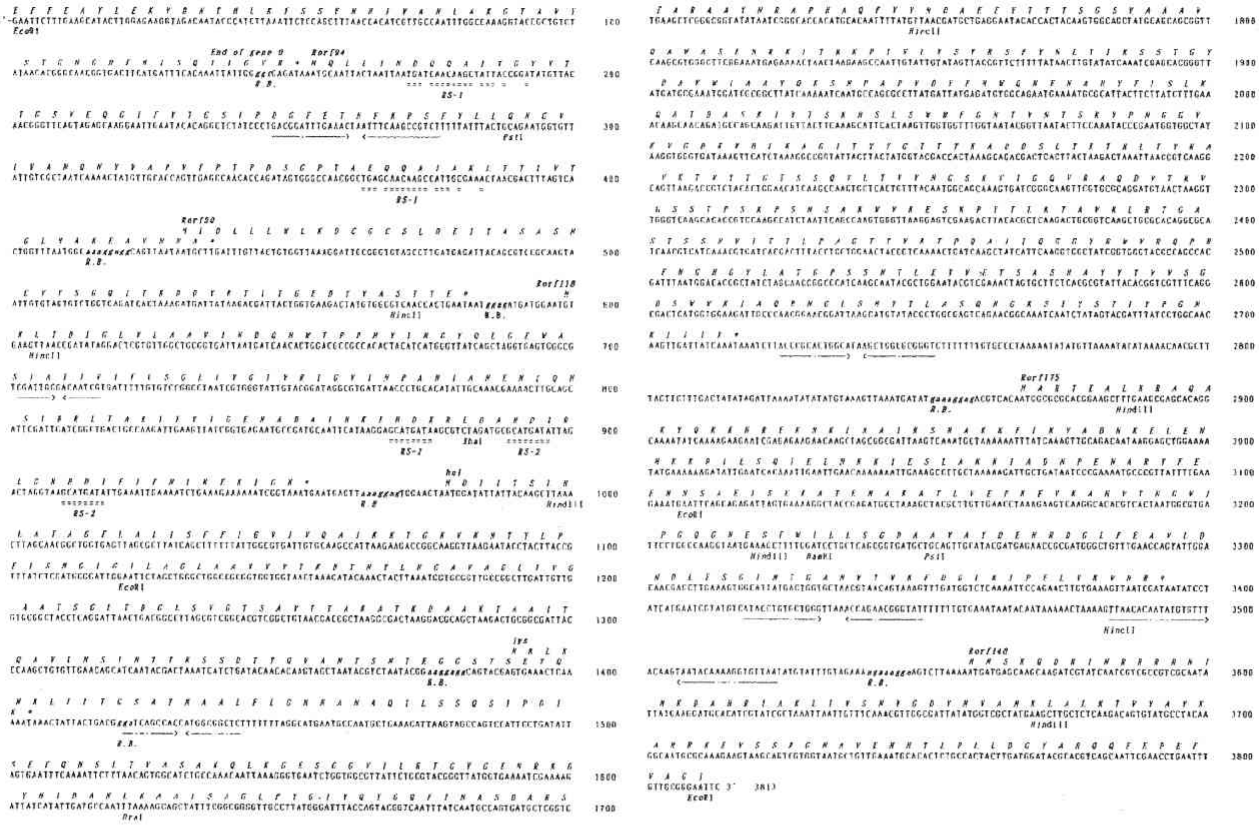


Fig. 2. DNA and predicted amino acid sequences of genes *0*, *Rorf94*, *Rorf50*, *Rorf118*, hol, lys, *Rorf175*, and *Rorf148*. Asterisks indicate stop codons. Arrows show inverted repeats. Bold italic lower letters indicate putative ribosome binding sites (R.B.). Dotted double lines present repeated sequences (RS). Restriction sites using the construction of the recombinant plasmids are shown as well.

3.3. Structural characteristics of the φg1e holin

φg1e holin shows high sequence similarity with other several predicted holins of phages (or monocins) from *Listeria*, which are thought to function as a second phage lysis protein (Zink et al., personal communication): e.g. more than 20 residues in a 58-aa region of monocin holin (Zink et al., 1995) are identical to those of φg1e holin (Fig. 4B).

Generally, holins encoded by phages are thought to be different in aa sequence, but their physical natures are predicted to be quite similar (Birkeland, 1994; Young, 1992). In the lambda system, the holin encoded by gene S is estimated to form lesions in the cytoplasmic membrane, through which the lysin encoded by gene R can be efficiently released to the periplasm (for a review, see Young, 1992).

Like lambda, φg1e contains a pair of hydrophobic transmembrane-spanning domains of about 20 residues separated by a highly charged sequence, albeit different in aa sequence. The structural similarity between the potential holins from the phages φg1e, mv1 (for a paper see Young, 1992), and φadh (Henrich et al., 1995) is presented in Fig. 4A; a sequence comparison of holins between φg1e, monocin (Zink et al., 1995), and φLC3 is shown in Fig. 4B.

3.4. Expression of the φg1e lysis genes in E. coli

To elucidate properties of the φg1e lytic system, various DNA regions containing *hol* and/or *lys* were cloned into *E. coli* expression vector (pUC18, pUC118 or pUC119) under p_{lac} control, and expressed in *E. coli* XL1-blue. Physical maps of these recombinant plasmids are shown in Table 1 and Fig. 1: pL119PBL (containing *Rorf50-Rorf118-hol-lys*); pL119XBL (*hol-lys*); pL118EHL (*lys*); pL119XSuL (*hol*). XL1-blue cells carrying pL119PBL grow at low temperature, but they can not multiply above 20 °C even without IPTG. XL1-blue bacteria harboring the recombinant, pL119XBL, pL118EHL, or pL119XSuL, can not grow above 25 °C, and the culture turbidity decreased significantly after a temperature shift up from 25 to 37 °C (Fig. 6A). The decrease of the cellular turbidity caused by pL119XBL occurred at 90 min after the temperature shift up. pL118EHL and pL119XSuL also induced turbidity decrease after approximately 240 min at 37 °C, although the process was slow and incomplete when compared

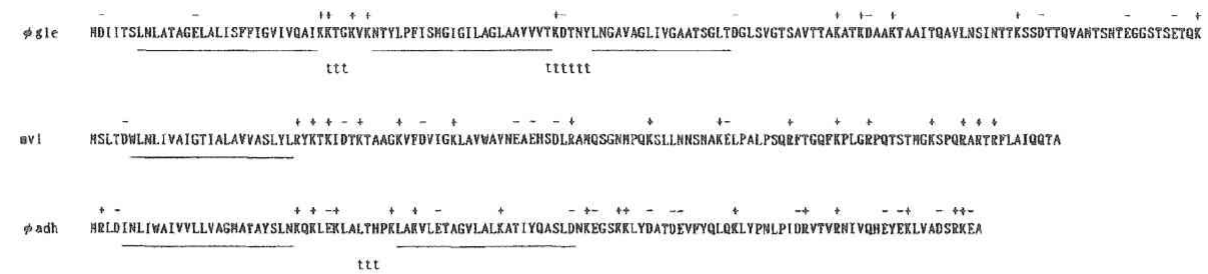
Fig. 3. Comparison of the φg1e lysin with presumptive muramidases encoded by other phages. Dashes indicate positions with conservative substitutions. (**A**) The N-termini of the lysins of the phage φg1e (this study), the *L. bulgaricus* phage mv1 (Boizet et al., 1990), the *L. gasseri* phage, φadh (Henrich et al., 1995), the *S. pneumoniae* phage Cp-1 (Garcia et al., 1990), and the *E. coli* phage lambda (Sanger et al., 1982), and the muramidase of fungus *Chalaropsis* (Fouche and Hash, 1978). The aa residues conserved through the lysins are boxed. (**B**) The middle regions of the lysins of the phages φg1e, mv1, φadh, Cp-1, and *L. lactis* phage φLC3 (Birkeland, 1994). The aa sequence and motifs A, B, C, and D are from Henrich et al. (1995). Consensus indicates the conserved aa residues; capital letters are conserved through the lysins. (**C**) C-termini of the lysins of the phages φg1e and φLC3. Double colons represent the identical aa residues. Consensus shows conserved aa residues (Birkeland, 1994). The homologous aa sequences between φg1e and φLC3 (Birkeland, 1994) were boxed.

with pL119XBL. Preliminary Northern blot experiments have shown that transcripts of *hol* and/or *lys* of these recombinant plasmids accumulated at 37 °C, but not at 25 °C; this transcription at 37 °C seems to be due to a leakiness of $lacI^q$ control and/or a high copy number of the recombinant plasmid, suggesting that the decrease of cellular turbidity correlates well with the mRNA level of *hol* and/or *lys* (data not shown). This inference has been further supported by in vivo experiments using rifampicin and chloramphenicol, showing that both transcription and translation at 37 °C are required for the decrease of cellular turbidity (data not shown).

When induced by IPTG at 37 °C (Fig. 6A), each of the recombinants pL119XBL (*hol-lys*), pL119XSuL (*hol*), and pL118EHL (*lys*) led to a faster onset of turbidity decrease than that without IPTG; the rate of the decrease by *hol-lys* is more rapid than that by *hol* or *lys*, and the start of decrease by *hol* is earlier than that by *lys*, suggesting that holin can form lesions in the cell membrane, and can promote lysin-mediated cell lysis, as suspected in other holin-lysin systems of φLC3 (Birkeland, 1994) and φadh (Henrich et al., 1995) as well as lambda (Young, 1992).

In addition, several recombinants containing φg1e *hol* and/or *lys*, which are free from p_{lac} control, were constructed (Table 1 and Fig. 1): pL119PPR (containing *Rorf50-Rorf118-hol-lys*); pL18DPR (*Rorf50-Rorf118-hol*); pL18DPRdel (*hol*); pL18EPR (*Rorf50-Rorf118*); pL118EHiL (*Rorf94*). pL18DPRdel was derived from pL18DPR, and is missing a 36-bp *Hin*cII fragment extending from 3′-terminus of *Rorf50* to 5′-terminus of *Rorf118*. XL1-blue harboring each of these recombinants grew stably at 30 °C, but not at 37 °C (Fig. 6B). Thus, pL119PPR exhibited an onset of turbidity decrease at 90 min after a temperature shift from 30° to 37 °C. pL18DPR induced the decrease after approximately 180 min at 37 °C, albeit incomplete when compared with pL19PPR. On the other hand, pL18DPRdel did not reduce the cellular turbidity after the temperature shift up, but inhibited the cell growth significantly. A φg1e

(A)



(B)

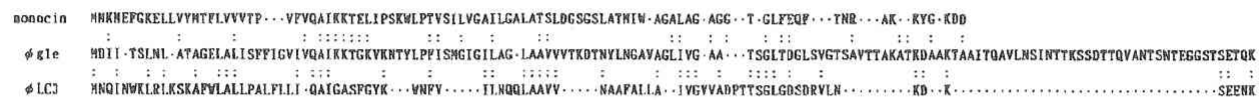


Fig. 4. Comparison of the φgle *hol* protein with presumptive holins encoded by other phages. +, basic aa; −, acidic aa. (A) Physical similarity between the holins of the phage φgle (this study), *L. bulgaricus* phage mv1 (Boizet et al., 1990), and *L. gasseri* phage φadh (Henrich et al., 1995). Possible transmembrane domains are indicated by solid bars, and potential beta-turn regions are shown by lower case t's (Young, 1992). (B) Sequence homology between the holins of the phage φgle (this study), *Listeria* monocin (Zink et al., 1995), and the *L. lactis* phage φLC3 (Birkeland, 1994). Double colons represent the identical aa residues. Dashes indicate positions with conservative substitutions.

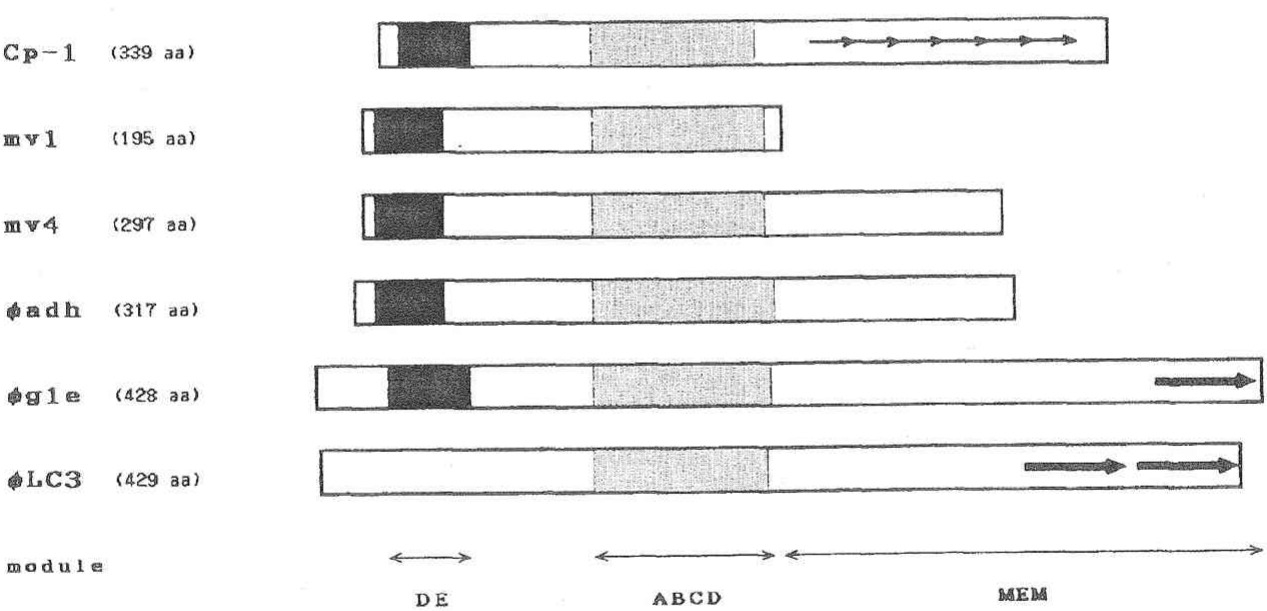


Fig. 5. Modules of the muramidases encoded by phages. The structures of the putative lysins of the phage φgle (this work), the *L. bulgaricus* phage mv1 (Boizet et al., 1990) and mv4 (Dupont et al., 1993), the *L. gasseri* phage φadh (Henrich et al., 1995), the *L. lactis* phage φLC3 (Birkeland, 1994), and the *S. pneumoniae* phage Cp-1 (Garcia et al., 1990) are compared. Three modules DE, ABCD, and MEM are shown in Fig. 3. The closed boxes show module DE. In φLC3 lysin, the two conserved residues Asp and Glu were not found in its N-terminus. The hatched boxes represent module ABCD (from Henrich et al., 1995). The six thin arrows in Cp-1 (Garcia et al., 1990) and the two thick arrows in φLC3 (Birkeland, 1994) indicate repeated sequences. The arrow in φgle shows a region similar to that of φLC3 (see Fig. 3).

DNA region preceding *hol* may contain a transcriptional promoter(s) functioning in *E. coli*.

Computer-assisted analysis detected several promoter-like sequences (-35 and -10 sequences) within *Rorf94* and *hol*, which are similar to the presumptive promoters found in *Lactobacillus* (for a review see Pouwels and Leer, 1993) and *E. coli* (Harley and Reynolds, 1987). Alternatively, gp*Rorf50* and/or gp*Rorf118* may accelerate the turbidity decrease in combination with holin and/or lysin, as in the coliphage P2 (Ziermann et al.,

TABLE I
Plasmids

Plasmid	Genotype or construct	Source or reference
pUC18	*Ap*^{r}, P_{lac} *lacZ'*	Vieira and Messing, 1987
pUC118	*Ap*^{r}, P_{lac} *lacZ'* M13 origin	Vieira and Messing, 1987
pUC119	*Ap*^{r}, P_{lac} *lacZ'* M13 origin	Vieira and Messing, 1987
pL119PBL	pUC119::2,938-bp *Pst*I-*Bam*HI fragment from ϕg1e (*Rorf50, Rorf118, hol* and *lys* under P_{lac})	This work
pL119XBL	pUC119::2,338-bp *Xba*I-*Bam*HI fragment from pL119PPR (*hol* and *lys* under P_{lac})	This work
pL118EHL	pUC118::1,760-bp *Eco*RI-*Hin*dIII fragment from pL119PPR (*lys* under P_{lac})	This work
pL119XSuL	pUC119::530-bp *Xba*I-*Sau*3AI fragment from pL119PPR (*hol* under P_{lac})	This work
pL118XDL	pUC118::732-bp *Xba*I-*Dra*I fragment from pL119PPR (*hol* and *Δlys-ΔlacZ'* under P_{lac})	This work
pL118EDL	pUC118::502-bp *Eco*RI-*Dra*I fragment from pL118XDL (*Δlys-ΔlacZ'* under P_{lac})	This work
pL118EHiL	pUC118::569-bp *Eco*RI-*Hin*cII fragment from ϕg1e (*Rorf94* under P_{lac})	This work
pL119PPR	pUC119::2,963-bp *Pst*I fragment from ϕg1e	This work
pL18DPR	pUC18::1,332-bp *Pst*I-*Dra*I fragment from pL119PPR	This work
pL18DPRdel	pUC18::1,296-bp *Pst*I-*Dra*I (*Δ*36-bp *Hin*cII) fragment from pL118DPR	This work
pL18EPR	pUC18::830-bp *Pst*I-*Eco*RI fragment from pL119PPR	This work

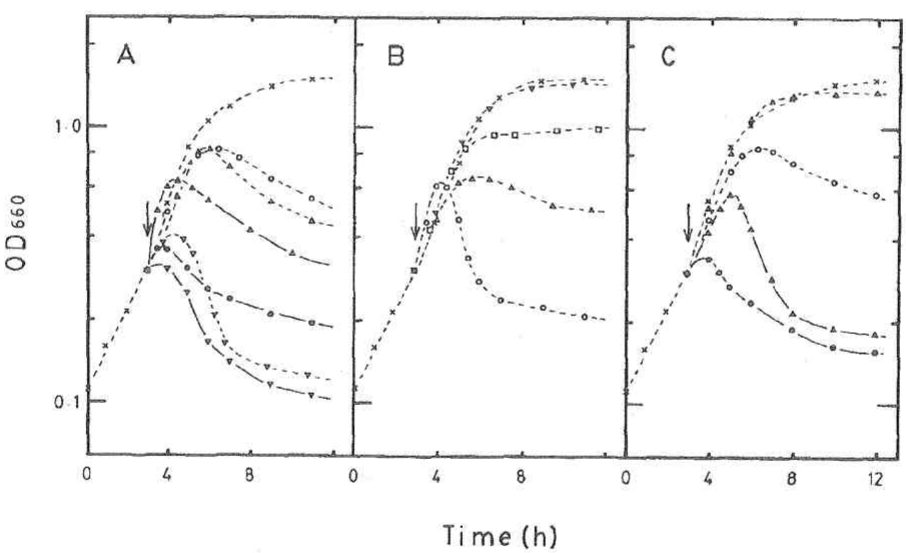


Fig. 6. Expression of the ϕg1e lysis genes in *E. coli*. (A) *E. coli* XL1-blue carrying the plasmid pL119XSuL (○, ●), pL118EHL (△, ▲), pL119XBL (▽, ▼) or the vector plasmid pUC118 or 119 (×), was grown at 25 °C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.3, the growth temperature was shifted to 37 °C with (open symbols) or without (filled symbols) concomitant addition of IPTG (final concentration 1 mM). Growth of the cells was monitored by measuring the change in A_{660}. (B) *E. coli* XL1-blue carrying the plasmid pL119PPR (○), pL18DPR (△), pL18DPRdel (□), pL18EPR (▽) or the vector plasmid pUC18 or 119 (X), was grown at 25 °C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.3, the growth temperature was sifted to 37 °C, and the cell growth was monitored as indicated in (A). (C) *E. coli* XL1-blue carrying the plasmid pL118XDL (○, ●), pL118EDL (△, ▲) or the vector plasmid pUC118 (X), was grown at 25 °C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.3, the growth temperature was shifted to 37 °C with (open symbols) or without (filled symbols) concomitant addition of IPTG (final concentration 1 mM), and the cell growth was monitored as indicated in (A).

1994). On the other hand, XL1-blue harboring pL18EPR or pL118EHiL grew normally, suggesting that each of gp*Rorf50*, gp*Rorf118* and gp*Rorf94* can not disturb the cell envelope integrity for oneself.

Two recombinant plasmids pL118XDL and pL118EDL have a fused gene (under p_{lac} control), termed DE/α, which is composed of 78-aa N-terminus of lysin and 91-aa C-terminus of *lacZ'* α-fragment

(Table 1 and Fig. 2). Upon temperature shift up from 30° to 37 °C in the absence of IPTG, pL118XDL carrying both of *hol* and *DE/α* manifested the turbidity decrease after 240 min (Fig. 6C), although the rate was somewhat low when compared with pL119XBL (*hol-lys*) (Fig. 6A), whereas pL118EDL harboring *DE/α* did not reduce the cellular turbidity (Fig. 6C). When induced by IPTG at 37 °C, both of the recombinants exhibited a rapid onset of turbidity decrease: *hol-DE/α*, within 60 min; DE/α, within 120 min. These results suggest that the module DE of ϕg1e lysin localized in its N-terminus (Fig. 3A and Fig. 5) contains an active center(s) for its lytic activity.

To estimate whether the ϕg1e holin-lysin system can make a lesion(s) in *E. coli* cell wall (or membrane), release of a cytoplasmic enzyme β-galactosidase was measured at 37 °C along with the turbidity drop of *E. coli* CK111 *lacZ*$^+$ carrying pL119PPR (*Rorf50-Rorf118-hol-lys*), which are free from *laqI*^{q} control. As presented in Fig. 7, β-galactosidase activity was found in the medium (120 min after the temperature shift), and its release from the cytoplasm correlated well with the rate of turbidity reduction.

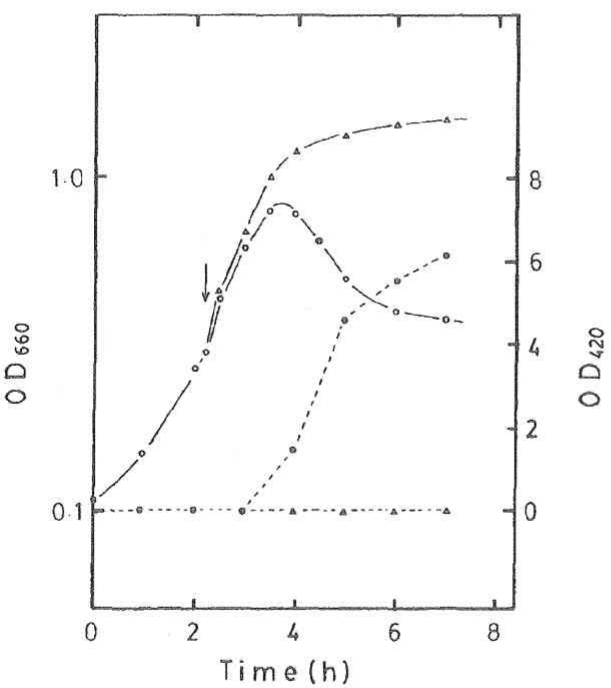


Fig. 7. Release of β-galactosidase by the ϕg1e lysis system. *E. coli* CK111 carrying the plasmid pL19PPR (○) or the vector plasmid pUC19 (△) was grown at 25 °C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.3, the growth temperature was shifted to 37 °C, and the cell growth was monitored as indicated in Fig. 6. At intervals, a potion of the culture was removed, and the cell-free supernatant fluid was obtained by centrifugation. β-Galactosidase activity of the supernatant of the cells harboring pL19PPR (●) or the vector pUC19 (▲) was monitored at A_{420} as described in Section 2.

These observations in *E. coli* suggested that the two ϕg1e putative proteins, holin and lysin, can introduce a lesion(s) in the *E. coli* cell envelope, and the holin-lysin system is essentially involved in the cytolytic process. As in lambda (Young, 1992) and P2 (Ziermann et al., 1994), the ϕg1e genes *hol* and *lys* might be under a complicated control. Further studies on structure, expression, function, and enzymatic properties of the ϕg1e lysis genes are now in progress.

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